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NEW UNITED STATES UTILITY PATENT APPLICATION

under 37 C.F.R. 1.53(b)

Atty. Docket No. 5538/1010.

Assistant Commissioner of Patents
Box Patent Applications
Washington, D.C. 20231

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Enclosed herewith is a new patent application and the following papers:

First Named Inventor (or application identifier): Mark Larche & Anthony B. Kay
Title of Invention: **METHODS AND COMPOSITIONS FOR DESENSITISATION**

1. ■ Specification 95 pages (including specification, claims, abstract) / 32 claims (17 independent)
   ■ **Insert on Page 1 of the of the specification –"This is a continuation of PCT/GB99/00080, filed January 11, 1999"** ____ _____.
2. ■ Declaration/Power of Attorney is:
   ■ attached in the regular manner (unexecuted).
   ☐ NOT included, but deferred under 37 C.F.R. § 1.53(f).
3. ■ 16 Distinct sheets of ☐ Formal ■ Informal Drawings
4. ☐ Preliminary Amendment.
5. ☐ Information Disclosure Statement
   ☐ Form 1449
   ☐ A copy of each cited prior art reference
6. ☐ Assignment with Cover Sheet.
7. ☐ Priority is hereby claimed under 35 U.S.C. § 119 based upon the following application(s):

Country	Application Number	Date of Filing (day, month, year)
GB	9800445.0	9/1/98
GB	9820474.6	21/9/98

☐ Priority is hereby claimed under 35 U.S.C. § 120 based upon the following application(s):

Country	Application Number	Date of Filing (day, month, year)
US	PCT/GB99/00080	11/1/99

8. ☐ Priority document(s).
9. ■ Statement Claiming Small Entity Status.

NEW UNITED STATES UTILITY PATENT APPLICATION
under 37 C.F.R. 1.53(b)

10. ☐ Microfiche Computer Program (Appendix).

11. ☐ Nucleotide and/or Amino Acid Sequence Submission.
- ☐ Computer Readable Copy.
- ☐ Paper Copy (identical to computer copy).
- ☐ Statement verifying identity of above copies.

12. Calculation of Fees:

FEES FOR	EXCESS CLAIMS	FEE	AMOUNT DUE
Basic Filing Fee (37 C.F.R. § 1.16(a))			$790.00
Total Claims in Excess of 20 (37 C.F.R. § 1.16(c))	12	22.00	$264.00
Independent Claims in Excess of 3 (37 C.F.R. § 1.16(b))	14	82.00	$1,148.00
Multiple Dependent Claims (37 C.F.R. § 1.16(d))	Y	270.00	$270.00
Subtotal - Filing Fee Due			$2,472.00
	REDUCE BY (%) | ($)		
Reduction by 50%, if Small Entity (37 C.F.R. §§ 1.9, 1.27, 1.28)	0.50		$1,236.00
TOTAL FILING FEE DUE			$0.00
Assignment Recordation Fee (if applicable) (37 C.F.R. § 1.21(h))	0	40.00	$0.00
GRAND TOTAL DUE			**$1,236.00**

13. PAYMENT is:
- ■ included in the amount of the GRAND TOTAL by our enclosed check. A general authorization under 37 C.F.R. § 1.25(b), second sentence, is hereby given to credit or debit our Deposit Account No. 19-0733 for the instant filing and for any other fees during the pendency of this application under 37 C.F.R. §§ 1.16, 1.17 and 1.18.
- ☐ not included; will be paid with response to Notice of Missing Parts.

14. All correspondence for the attached application should be directed to:

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15. Other: _____

Date: July 3, 2000

By: Kathleen Williams
Kathleen M. Williams
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant or Patentee:	Mark Larche and Anthony B. Kay
Serial No.:	Not Assigned Yet {A Continuation of PCT/GB99/00080 filed January 11, 1999}
Filed:	Concurrent Herewith
Entitled:	METHODS AND COMPOSITIONS FOR DESENSITISATION
Attorney Docket No.:	5538/1010

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS

(37 CFR 1.9(d) AND 1.27(d)) -SMALL BUSINESS CONCERN

I hereby declare that I am an official empowered to act on behalf of the small business concern identified below:

Name of Organization: CIRCASSIA LIMITED

Address of Organization: 90 Fetter Lane, London EC4A 1JP

UNITED KINGDOM

I hereby declare that the small business concern identified above qualifies as a small business concern as defined in 37 C.F.R. § 1.9(d) for purposes of paying reduced fees under § 41(a) and (b) of Title 35, United States Code, with regard to the invention entitled

by inventor(s) Mark Larche and Anthony B. Kay

described in

[X] the specification filed herewith

[ ] Application Serial No. _____, filed _____.

[ ] Patent No. _____, issued _____.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern with regard to the above-identified invention.

If the rights held by the above identified small business concern are not exclusive, each individual concern or organization having rights in the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR § 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR § 1.9(d), or a nonprofit organization under 37 CFR § 1.9(e).

_____

_____
TITLE IN ORGANIZATION

90 Fetter Lane, London E4A 1JP , United Kingdom
ADDRESS

_____
SIGNATURE

_____
DATE

PATENT 5538/1010

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Mark Larche and Anthony B. Kay

Serial No.: Not Yet Assigned

Filed: Concurrent Herewith
[A Continuation of PCT/GB99/00080 filed Januay 11, 1999]

Entitled: METHODS AND COMPOSITIONS FOR DESENSITISATION

Assistant Commissioner for Patents & Trademarks
Washington, D.C. 20231

Peliminary Amendment

Sir:

Kindly preliminarily amend the specification and claims as follows:

In the specification: Insert on page 1 of the specification: **"This is a continuation of PCT/GB99/00080, filed January 11, 1999. _____ _____.**

In the claims:

Cancel claims 13 and 14.

Amend claim 4 by deleting "any one of Claims 1 to 3" and substituting therefore – claim 1--.

Amend claim 5 by deleting "any one of Claims 1 to 4" and substituting therefore – claim 1--.

Amend claim 15 by deleting "any one of claims 1 to 5" and substituting therefore –claim 1—and by deleting "or a use according to claim 13 or 14".

Amend claim 16 by deleting "or a use according to claim 13 or 14".

Amend claim 19 by deleting "or 19".

Amend claim 20 by deleting "any one of claims 17 to 19" and substituting therefore – claim 17-.

Amend claim 21 by deleting "any one of claims 17 to 20" and substituting therefore – claim 17-.

Amend claim 22 by deleting "any one of claims 17 to 21" and substituting therefore – claim 17-.

Amend claim 23 by deleting "any one of claims 17 to 22" and substituting therefore – claim 17-.

Amend claim 24 by deleting "any one of claims 17 to 23" and substituting therefore – claim 17-.

Amend claim 30 by deleting "or 29".

Amend claim 31 by deleting "any one of claims 28 to 30" and substituting therefore – claim 28-.

Respectfully submitted,

7/5, 2000
Dated

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METHODS AND COMPOSITIONS FOR DESENSITISATION

The present invention relates to methods and compositions for desensitising patients who are hypersensitive to particular allergens, especially polypeptide allergens. Moreover, the invention relates to immunological vaccines which may be used to prevent and/or treat conditions involving hypersensitivity to allergens.

The ability of the immune system to elicit a response to a particular molecule depends critically upon its ability to recognise the presence of an antigen. Classically, the term antigen has been associated with the ability of a molecule to be an antibody generator *via* induction of B-cells. It is now known, however, that T cells also possess the ability to recognise antigens. T-cell antigen recognition requires antigen presenting cells (APCs) to present antigen fragments (peptides) on their cell surface in association with molecules of the major histocompatibility complex (MHC). T cells use their antigen specific T-cell receptors (TCRs) to recognise the antigen fragments presented by the APC. Such recognition acts as a trigger to the immune system to generate a range of responses to eradicate the antigen which has been recognised.

T lymphocytes have been implicated in the pathogenesis of a wide variety of diseases involving immune recognition of antigens derived both from the internal (host) and external environments. Autoimmune diseases such as autoimmune thyroiditis, rheumatoid arthritis and lupus erythrematosus arise from the recognition by the immune system of host, or self, antigens.

Recognition of external antigens by the immune system of an organism, such as man, can in some cases result in diseases, known as atopic conditions. An example of the latter are the allergic diseases including

other foreign antigens or triggering an allergic response itself would be of great benefit to allergic individuals.

Asthma can be provoked by inhalation of allergen in the clinical laboratory under controlled conditions. The response is characterised by an early asthmatic reaction (EAR) followed by a delayed-in-time late asthmatic reaction (LAR) (See *Allergy and Allergic Diseases* (1997), A.B. Kay (Ed.), Blackwell Science, pp 1113 to 1130). The EAR occurs within minutes of exposure to allergen, is maximal between 10 and 15 min and usually returns to near baseline by 1 hour. It is generally accepted that the EAR is dependent on the IgE-mediated release of mast cell-derived mediators such as histamine and leukotrienes. In contrast the LAR reaches a maximum at 6-9 hours and is believed to represent, at least in part, the inflammatory component of the asthmatic response and in this sense has served as a useful model of chronic asthma.

The late asthmatic response is typical of responses to allergic stimuli collectively known as late phase responses (LPR). LPR is seen particularly in the skin and the nose following intracutaneous or intranasal administration of allergens.

Using cat allergic individuals (rhinitic and asthmatic), Norman *et al* (1996) *Am. J. Respir. Crit. Care Med.* 154:1623-8 attempted to induce the counterpart of murine experimental T cell tolerance by subcutaneous injection of "T cell reactive peptides" (termed IPC1 and IPC2) in humans. Peptides were designed on the basis of patterns of epitope recognition of short overlapping peptides by Fel d 1 reactive T cell lines. It was found that peptides derived from chain 1 gave greater proliferative responses than chain 2, with the majority of activity being associated in the N terminal region of chain 1. IPC1 and IPC2 were considerably longer (27

We have observed that peptide allergens used in immunotherapy associate with particular MHC types in patients. Moreover, successful desensitisation of patients is achieved where a peptide allergen is used which is capable of giving an initial LPR in an individual to whom it is administered.

The MHC complex is a genetic locus made up of a number of genes which encode MHC molecules. MHC molecules are also known as Human Leucocyte Antigens (HLA).

Each individual inherits a number of MHC genes from each parent and the genes are referred to collectively as the individual's haplotype. This is a genetic term referring to the genes rather than the molecules they encode. Although the term "haplotype" should, strictly speaking, be used to describe the genes inherited from one parent, it is generally used to include genes from both sets of parents. Where the term is used in this patent specification it is given this general meaning unless the context suggests the stricter meaning.

A first aspect of the invention provides a method of desensitising a patient to a polypeptide allergen the method comprising administering to the patient a peptide derived from the allergen wherein restriction to a MHC Class II molecule possessed by the patient can be demonstrated for the peptide and the peptide is able to induce a late phase response in an individual who possesses the said MHC Class II molecule.

Restriction to a MHC Class II molecule possessed by the patient can be demonstrated for the peptide by, for example, T cell reactivity to the peptide. By "MHC Class II molecule possessed by the patient" is meant

It will be appreciated that the peptide may conveniently be blocked at its N- or C-terminus so as to help reduce susceptibility to exoproteolytic digestion.

By "restriction to a MHC Class II molecule possessed by the patient can be demonstrated for the peptide" we mean that the peptide is able to bind to a particular MHC Class II possessed by the patient. That is not to say that a particular peptide cannot bind to another MHC Class II molecule. Peptides are generally only recognised in the context of a "self" MHC molecule, thus recognition of MHC-bound peptides by an individual's T cells is generally restricted by the MHC molecules expressed by the individual molecule.

Although binding to the given MHC Class II molecule may be demonstrated directly using suitable samples from the patient, whether or not a particular peptide can bind to a particular MHC Class II molecule (ie is restricted by a particular Class II molecule) can readily be determined *in vitro* using methods well known in the art, some of which are disclosed below.

Determination of the MHC Class II haplotype of the patient or the identification of particular MHC Class II genes possessed by the patient can readily be determined using any suitable method as is well known in the art, including the PCR-based methods described more fully below for example techniques based on those of Olerup & Zetterquist (1992) *Tissue Antigens* 29:225-235. Determination of the MHC Class II haplotype indicates which MHC molecules are expressible by an individual.

By "late phase response" we include the meaning as set forth in *Allergy*

However, they may be derived from only portions of the polypeptide allergen such that some portions of the polypeptide allergen are not represented in the plurality of peptides (for example, as is shown below, some peptides derived from an allergen may not be very soluble in aqueous solution and so may not be useful and other peptides may not show restriction to MHC Class II molecules). MOPs or any peptides derived from the allergen and present in the composition can be designed by reference to the amino acid sequence of the polypeptide allergen. Typically, the peptides are at least seven amino acid residues. Typically, the peptides would be between around 14 to 18 amino acid residues in length. It is preferred that the peptides have a reduced ability to bind IgE compared to longer peptides containing the same sequence. It is particularly preferred if the peptides are substantially incapable of binding IgE. Typically, when the MOPs overlap, the overlap is around one amino acid residue. This is particularly useful when the MOPs are used in *in vitro* T cell assays in order to identify MHC-binding peptides which may then be screened for their ability to induce LPR in an individual. More details of screening procedures are given below.

MHC Class II molecules are encoded by MHC Class II genes. There are at least three loci (DR, DQ and DP) that encode MHC Class II molecules, and each individual has two copies of each locus. These loci exhibit considerable genetic diversity and the preponderance of different MHC Class II genes (alleles) varies. The approximate frequencies of various MHC Class II genes (alleles) from a normal (disease free) population of people in England is described in Haworth S, Sinnott P, Davidson J & Dyer P. Caucasian England Normal In: HLA Typing 1997, Eds: Terasaki, PI and Gjertson, DW, Publishers: UCLA tissue typing laboratory, incorporated herein by reference.

the patient includes peptides for which restriction to the MHC Class II DQ molecules DQB1*0301 and DQB1*0601 can be demonstrated. In a further embodiment it is preferred if the plurality of peptides further includes peptides for which restriction to any one or more of the MHC Class II DQ molecules DQB1*0201, DQB1*0501 and DQB1*0602 can be demonstrated.

It is preferred if the plurality of peptides includes only a single peptide for which restriction to a particular MHC Class II molecule can be demonstrated.

Restriction to a particular Class II molecule can be demonstrated as has been described above and is described in more detail below. It will be appreciated that it may not be possible to derive a peptide for which restriction to a particular Class II molecule can be demonstrated; for example, a particular polypeptide allergen may not contain a T cell epitope which can be presented by every MHC Class II molecule. In this case, of course, such a peptide is not present in the plurality of peptides derived from the polypeptide allergen.

By "desensitising a patient to a polypeptide allergen" is meant inhibition or dampening of allergic tissue reactions induced by allergens in appropriately sensitised individuals. It will be appreciated that whether or not a patient is sensitive to a particular polypeptide allergen can be assessed using well known procedures such as skin prick testing with solutions of allergen extracts, induction of cutaneous LPRs, clinical history, allergen challenge and radio-allergosorbent test (RAST) for measurement of allergen specific IgE, and whether or not a particular patient is one who is expected to benefit from treatment may be determined by the physician based, for example, on such tests.

Preferably, at least one peptide is present in the composition for which restriction to each of MHC Class II DP molecules DPB1*0201, DPB1*0301, and DPB1*0401 can be demonstrated.

Preferably, at least one peptide is present in the composition for which restriction to each of MHC Class DQ molecules DQB1*0301 and DQB1*0601 can be demonstrated. In a further embodiment it is preferred if the plurality of peptides further includes peptides for which restriction to any one or more of the MHC Class II DQ molecules DQB1*0201, DQB1*0501 and DQB1*0602 can be demonstrated.

These preferences are all with the proviso that for any particular allergen it may not be possible to derive a peptide for which restriction to a particular Class II molecule can be demonstrated.

Although the composition (or a peptide within the composition) is able to induce a LPR in an individual possessing the given MHC Class II molecule (and as described below in more detail suitable compositions and peptides may be identified by their ability to induce a LPR), it should be appreciated that when the composition (or a peptide within the composition) is used to treat a patient it is preferable that a sufficiently low concentration of the composition or peptide is used such that no observable LPR will occur but the response will be sufficient to partially desensitise the T cells such that the next (preferably higher) dose may be given, and so on. In this way the dose is built up to give full desensitisation but often without ever inducing a LPR in the patient (although, of course, the composition or peptide is able to do so at a higher concentration than is administered. It will be appreciated further, and as discussed in more detail below, induction of LPR in an individual

allergen-derived peptide components a sufficient number of peptides, each of which demonstrate restriction to a particular MHC Class II molecule and which are able to induce a LPR in an individual who possesses the said MHC Class II molecule, such that for at least 75% of the population a peptide is present in the composition which is MHC Class II restricted and which is capable of inducing a LPR in an individual with an appropriate restricted MHC Class II molecule.

It is well known that the frequency of particular MHC Class II molecules in a population varies with ethnic groups, and that for at least some ethnic groups the frequency of particular MHC Class II molecules is known (see, for example, HLA Typing 1997, supra). For example, the frequency of particular MHC Class II molecules is different in the Caucasian population compared to the Mongoloid population or Negroid population and so on. It will readily be appreciated that the polypeptide allergen-derived peptides to be included in a composition of the invention may be selected according to the ethnic group to which the patient belongs. For example, compositions of the invention may readily be prepared for desensitisation to a particular polypeptide allergen by reference to the MHC Class II gene frequencies in the Caucasian or Mongoloid or Negroid populations.

A third aspect of the invention provides a composition of the second aspect of the invention packaged and presented for use in medicine. In particular, the composition will be packaged and presented with an indication of who may be treated (in particular who may benefit from being treated) with the composition including, if desirable, an indication of the MHC Class II molecules to which the peptides within the composition are restricted.

It will be appreciated that the composition of the second aspect of the

least 75% (or more preferably 80%, or 85% or 90%) of the population a peptide is present in the composition which is MHC Class II restricted and which is capable of inducing a late phase response in an individual with an appropriate restricted MHC Class II molecule, then it may not be necessary or desirable to type the patient to determine which MHC Class II molecules he or she possesses.

The polypeptide allergen may be any polypeptide allergen, some of which are described in more detail below.

A seventh aspect of the invention provides a method of selecting a peptide for use as an immunotherapeutic agent for desensitising a patient to a polypeptide allergen capable of eliciting an allergic response in the patient, which patient possesses a particular MHC Class II molecule, the method comprising the steps of (1) selecting a candidate peptide derived from the polypeptide allergen, (2) determining whether the candidate peptide demonstrates restriction to the said MHC Class II molecule, and (3) determining whether the candidate peptide is able to induce a late phase response in an individual who possesses the said MHC Class II molecule.

The candidate peptide may be any peptide derived from the polypeptide allergen and is, conveniently, a polypeptide in the size range described elsewhere as being a suitable size of a peptide for use in immunotherapy.

Whether or not the candidate demonstrates restriction to the said MHC Class II molecule may be determined by any suitable method such as those well known in the art, some of which are described in the Examples.

Whether or not the candidate peptide is able to induce a LPR can be determined by the methods described herein and which are well known in

not be the patient.

In an eighth aspect, the invention provides a method for testing for candidate peptides for further selection according to the preferred embodiment discussed immediately above of the invention, comprising the steps of:

a) assaying a peptide or peptides in a T-cell activation assay and selecting peptides capable of inducing activation of an individual's T-cells;

b) tissue-typing the individual to determine MHC type;

c) determining the MHC molecule(s) bound by each candidate peptide; and

d) selecting a peptide or peptides satisfying part (a) above and capable of binding to an MHC type possessed by the individual, for use as a candidate peptide in a method according to the preferred embodiment discussed immediately above.

In a ninth aspect, the invention provides a method for selecting a peptide for use as an immunotherapeutic agent for desensitising a patient to an allergen comprising the steps of:

a) tissue-typing the patient to determine MHC Class II type; and

b) selecting, from a database of peptides which are known to bind to particular MHC molecules and induce a late phase response in an individual possessing such MHC Class II molecules, one or more peptides capable of binding to the MHC Class II molecules possessed by the

bind within the peptide binding groove of certain MHC molecules and be subsequently displayed upon the surface of antigen presenting cells. If the peptide/MHC complexes are present upon the antigen presenting cell surface in sufficient numbers they may then activate T cells which bear the appropriate peptide/MHC-specific T cell receptors.

Due to the polymorphic nature of the MHC, individuals in an outbred population such as man will express different combinations of MHC molecules on their cell surfaces. Since different MHC molecules can bind different peptides from the same molecule based on the size, charge and shape of the peptide, different individuals will display a different repertoire of peptides bound to their MHC molecules.

Identification of universal MHC-binding peptide epitopes in an outbred population such as man is more difficult than in inbred animals (such as certain strains of laboratory mice). On the basis of differential MHC expression between individuals and the inherent differences in peptide binding and presentation which this brings, it is unlikely that a single peptide can be identified which will be of use for desensitisation therapy in man for most diseases unless the association of a particular MHC molecule with that disease is very strong. For example, the HLA-B27 molecule has been shown to have a close relationship with ankylosing spondylitis, where approximately 90% of sufferers express HLA-B27. For some autoimmune diseases, certain disease HLA associations have been demonstrated eg HLA-DR4 and rheumatoid arthritis, but these associations are much weaker than for ankylosing spondylitis.

In allergic diseases, associations are even weaker if demonstrated at all. For this reason, it is unlikely that therapies centred around a single peptide (even an immunodominant one) or small numbers of peptides will be

suggested by the observation that cyclosporin A attenuated the LAR, but not the EAR, provoked by allergen inhalation (Sihra *et al* (1997) *Thorax* 52:447-452). Furthermore a single infusion of anti-CD4 produced significant improvement in lung function in chronic corticosteroid-dependent asthmatics. However it has been difficult to determine whether T cell activation, as an initiating event, leads directly to airway narrowing in asthmatic patients and therefore an asthmatic response.

As described herein, it has now been shown that T cells can be selectively activated, and then rendered unresponsive. Moreover the anergising or elimination of these T-cells leads to desensitisation of the patient for a particular allergen. The desensitisation manifests itself as a reduction in response to an allergen or allergen-derived peptide, or preferably an elimination of such a response, on second and further administrations of the allergen or allergen-derived peptide. The second administration may be made after a suitable period of time has elapsed to allow desensitisation to occur; this is preferably any period between one day and several weeks. An interval of around two weeks is preferred.

Based on these results, the invention provides a method for desensitising a patient to a polypeptide allergen which comprises the administration to the patient of a peptide specifically selected to induce LPR and subsequent desensitisation in the patient wherein the peptide is restricted by a particular MHC Class II molecule and capable of inducing LPR in an individual who possesses the given MHC Class II molecule to which the peptide is restricted. The peptides for desensitisation may be selected according to whether they induce LPR.—

LPR is defined as set forth in *Allergy and Allergic Diseases* (1997) A.B.

allergen, as may truncations from the N or C termini. Deletions and substitutions may moreover be made to the fragments of the allergen comprised by the invention. Peptides may be produced from a DNA which has been subjected to *in vitro* mutagenesis resulting eg in an addition, exchange and/or deletion of one or more amino acids. Preferably, peptides are produced by peptide synthesis according to known techniques using commercially available peptide synthesisers. Mutations and/or truncations may thus be made by changing the amino acid sequence during the synthesis procedure.

Suitable variants capable of binding to TCRs may be derived empirically or selected according to known criteria. Within a single peptide there are certain residues which contribute to binding within the MHC antigen binding groove and other residues which interact with hypervariable regions of the T cell receptor (Allen *et al* (1987) *Nature* 327:713-5). Within the residues contributing to T cell receptor interaction, a hierarchy has been demonstrated which pertains to dependency of T cell activation upon substitution of a given peptide residue. Using peptides which have had one or more T cell receptor contact residues substituted with a different amino acid, several groups have demonstrated profound effects upon the process of T cell activation. Evavold & Allen (1991) *Nature* 252:1308-10) demonstrated the dissociation of T cell proliferation and cytokine production. In this *in vitro* model, a T cell clone specific for residues 64-76 of haemoglobin (in the context of I-E^k), was challenged with a peptide analogue in which a conservative substitution of aspartic acid for glutamic acid had been made. This substitution did not significantly interfere with the capacity of the analogue to bind to I-E^k. Following *in vitro* challenge of a T cell clone with this analogue, no proliferation was detected although IL-4 secretion was maintained, as was the capacity of the clone to help B cell responses. In a subsequent study

domains. Preferably, smaller polypeptides derived from the allergen according to the invention define a single epitope of the allergen capable of binding a TCR. Fragments may in theory be almost any size, although smaller fragments are more likely to be restricted to a single MHC molecule and are thus preferred. Preferably, fragments will be between 5 and 50, preferably between 5 and 25, and advantageously about 17 amino acids in length. It is preferred if the peptides do not invoke an IgE response and do not lead to the release of histamine from enriched basophils or mast cell preparations from most sensitised individuals.

Candidate peptides potentially capable of inducing LPR in a patient may be preselected in order to maximise the chances of identifying a therapeutically useful peptide in *in vivo* tests. The steps of this aspect of the invention comprise the determination that the peptide is MHC Class II restricted, for example it is capable of causing T-cell proliferation when associated with an MHC molecule present in the patient to be treated. Thus, in a particular embodiment the selection procedure can be broken down into three steps, performed either sequentially (in any order) or together:

a) assaying a peptide or peptides in a T-cell activation assay and selecting peptides capable of inducing activation in an individual's T-cells;

b) tissue-typing the individual to determine MHC Class II type; and

c) determining the MHC Class II molecule bound by each candidate peptide.

Steps (a) and (c), in particular, may be combined in a single T-cell activation assay. Preferably, the assay involves the use of cells transfected

agent for desensitising a patient to an allergen comprising the steps of:

a) tissue-typing the patient to determine MHC Class II type; and

b) selecting, from a database of peptides which are known to bind to particular MHC Class II molecules and induce a late phase response in an individual possessing such MHC Class II molecules, one or more peptides capable of binding to the MHC Class II molecules possessed by the patient.

For the avoidance of doubt, the individual referred to in part (b) above need not necessarily be the same individual as the patient undergoing treatment whom is tissue typed in part (a). In fact, once the MHC Class II restriction of a particular allergen-derived peptide is determined, and it has been determined that the peptide is capable of inducing a LPR in an individual, particularly an appropriately sensitised individual, who possesses the said MHC Class II molecule, there is no requirement to test the ability of the patient's own MHC Class II molecules.

Allergens that may be amenable to desensitisation procedures as described herein include the peptides derived or chosen from the list comprising the allergens; Fel d 1 (the feline skin and salivary gland allergen of the domestic cat *Felis domesticus* - the amino acid sequence of which is disclosed in WO 91/06571), Der p I, Der p II, Der fI or Der fII (the major protein allergens from the house dust mite dermatophagoides - amino acid sequences disclosed in WO 94/24281).

The invention is applicable substantially to any allergen, including allergens present in any of the following: grass, tree and weed (including ragweed) pollens; fungi and moulds; foods eg fish, shellfish, crab lobster,

type.

The invention moreover provides a peptide listed in a database according to the invention, for use in therapy. Preferably, such peptides are useful in methods for desensitising patients to allergens in accordance with the methods set forth herein. Peptides to be included in the database, and peptides which may be useful either individually or as a mixture in a composition of the invention may readily be selected by the methods of the invention from polypeptide allergens whose polypeptide sequences, or reference to polypeptide sequences, are given in Example 6.

The MHC molecules expressed on APCs which bind peptides derived from a specific allergen may be identified by methods known in the art, such as T cell proliferation studies with MHC blocking antibodies, and PCR techniques, for example techniques based on those of Olerup & Zetterquist (1992) *Tissue Antigens* 29:225-235. Thus, antigen-presenting cells, expressing a variety of MHC molecules may be incubated with allergen and T cells and the latter observed for proliferation. Addition of antibodies to specific MHC classes may then be made in repeat incubations in order to identify the restricted MHC in respect of the allergen being tested. See Van Neerven RJJ *et al* (1994) *Immunol* 82:351-356, and Yssel H *et al* (1992) *J Immunol* 148:738-745.

Alternatively, cells presenting a single MHC Class II type, for example cells such as fibroblast cells transfected with the genes encoding an MHC Class II molecule, may be incubated with individual peptides for which T-cell clones or lines are known to be specific. Culturing of such T-cell clones or lines with peptide presented by the appropriate MHC Class II molecule will lead to T-cell proliferation. T cell proliferation is not the only indicator that a particular peptide binds to a particular MHC Class II

a measurable or observable LPR. Subsequent administration will lead to desensitisation of the patient. For example, if the peptide is that of SEQ. ID No. 3 (a fragment of the Fel d 1 allergen), then upon first administration of this peptide a LPR will be observed. Subsequent administration of this peptide results in a weaker reaction or no reaction, the patient having been desensitised.

The invention also relates to the use of a peptide in desensitising a patient against an allergen, the peptide being identified by its capability to bind to at least one MHC Class II molecule present in an individual and induce LPR in an individual who possesses the said MHC Class II molecule, wherein the patient also possesses the given MHC Class II molecule.

Peptides may be administered to a patient singly or in combination (for example as a composition as defined above). Thus, the database according to the invention may be used to prepare a designer vaccine which may be used to desensitise a patient to a chosen allergen, on the basis of the patient's MHC Class II type. The MHC Class II type can be correlated to the known MHC Class II binding characteristics of the peptides listed in the database, and the appropriate peptides selected and combined to form a designer vaccine. Similarly, the database may be used to design compositions (ie mixtures of peptides) which contain sufficient number of peptides, each of which demonstrate restriction to a particular MHC Class II molecule and which are able to induce a late phase response in an individual who possesses the said MHC Class II molecule, such that for at least 75% (preferably at least 80% or 85% or 90%) of the population a peptide is present in the composition which is MHC Class II restricted and which is capable of inducing a late phase response in an individual with the appropriate MHC Class II molecule.